Novel variant detection is essential when attempting to generically confirm the clinical diagnosis of complement-mediated Thrombotic Microangiopathies (TMA)

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Atypical Hemolytic Uremic Syndrome (aHUS):

- Complement-mediated TMA
- Genetic (multiple genes implicated)
- Progressive, life-threatening disease
- 50% die, ESRD or kidney damage in year 1 despite PE/PI
- Historically diagnosed by excluding other disease
- Most frequently occurs following a triggering event that reveals a defect in the complement regulation or coagulation system
aHUS: Clinical Presentation

- Another Thrombotic Microangiopathy (TMA)
- Presents like TTP
  - Perhaps 50% of cases are adults
  - Neurologic 50%
  - Diarrhea 30% (without STEC)
  - Renal may or may NOT be prominent initially
- May Relapse / Progress despite plasma exchange
- Multi-organ involvement
- **TTP with an ADAMTS-13 Activity >5-10%**
ADAMTS-13 Activity can be decreased within multiple disease states

aHUS: Genes Responsible

- Strongest evidence: CFH, MCP(CD46), CFI, C3, CFB, CFHR1, CFHR3, CFHR4, CFHR5, Thrombomodulin (THBD), DGKE, Plasminogen (PLG) – good agreement among labs offering test
- Incomplete story: currently available genetic tests likely to catch 70% of aHUS cases (‘rule in’ test)
- Increasing evidence: MASP-2, FKRP, ADAMTS-13, other complement and coagulation genes
Trending percentage of aHUS patients carrying detectable mutations over time.

- **Warwicker et al 1998**: First mutation described.
  - 1998
  - 74%
  - 3 genes

- **Noris et al 2010**: 9 genes
  - 2010

- **Bu et al 2014**: 86%
  - 2014
  - ~20 genes

- **Noris and Remuzzi 2005**: 40%
  - 2005
  - 3 genes

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ASFA Annual Meeting 2015, San Antonio
Survival in aHUS by genotype

Source: Noris et al. 2010 CJASN
Table 1 | Risk of aHUS recurrence according to the implicated genetic abnormality

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein location</th>
<th>Functional impact</th>
<th>Mutation frequency in aHUS (%)</th>
<th>Recurrence frequency after transplantation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFH</td>
<td>Plasma</td>
<td>Loss</td>
<td>20–30</td>
<td>75–90</td>
</tr>
<tr>
<td>CFI</td>
<td>Plasma</td>
<td>Loss</td>
<td>2–12</td>
<td>45–80</td>
</tr>
<tr>
<td>CFB</td>
<td>Plasma</td>
<td>Gain</td>
<td>1–2</td>
<td>100</td>
</tr>
<tr>
<td>C3</td>
<td>Plasma</td>
<td>Gain</td>
<td>5–10</td>
<td>40–70</td>
</tr>
<tr>
<td>MCP</td>
<td>Membrane</td>
<td>Loss</td>
<td>10–15</td>
<td>15–20</td>
</tr>
<tr>
<td>THBD</td>
<td>Membrane</td>
<td>Loss</td>
<td>5</td>
<td>1 case</td>
</tr>
</tbody>
</table>

Genetic polymorphism (frequency in control populations)

| Homozygous CFHR1del (3–8%) | Circulating | Undetermined | 14–23 (>90% in patients with anti-CFH antibodies) | NA |

Abbreviations: aHUS, atypical hemolytic uremic syndrome; C3, complement C3; CFB/H/I, complement factor B/H/I; MCP, membrane cofactor protein; NA, not available; THBD, thrombomodulin.
Test Methodology: aHUS genetic panel design:

- Fast and cheap (maximize clinical utility)
- Detect all known causative mutations and associated polymorphisms
- Include all genes associated with aHUS (sufficient evidence)
- Detect novel mutations (many case reports)
- Detect deletions (CFHR1-CFHR3 deletion and others)
- Allow for the rapid addition of new genes

- NGS presents best alternative
The Path to the Clinical Diagnosis of aHUS

Previous

- TMA
- Shiga toxin -
- ADAMTS-13 > 5%
- PI/PX?
- Treat?
- (3-14 days)

Current

- TMA
- Shiga toxin -
- ADAMTS-13 > 5%
- PI/PX?
- Treat?
- (24 hours)

- Clinical DX?
- Clinical DX?
- Clinical DX?
- Clinical DX?
- Clinical DX?
- Confirm DX?
- Complement Genetics
- Renal/Skin Biopsy (MAC)
- H, I, B Levels
- PI/PX ?
- Treat?
- (48 hours)
- (72 hours)
- (> 4 weeks)

Real-time genetic confirmation of a clinical diagnosis

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NGS methodology for aHUS genetic panel:

Genes Sequenced: 12 [CFH, MCP(CD46), CFI, C3, CFB, CFHR1, CFHR3, CFHR4, CFHR5, Thrombomodulin (THBD), DGKE, Plasminogen (PLG)]

Read Depth: Ave. 200-2,000 reads per base pair (min. 50)

Comparison Sequence: Human genome (Hg19) sequence

Multiple bioinformatic tools used to predict damage (novels)

Input quantity: 7.5ng of DNA (venous blood or buccal swab)

TAT: 2 – 5 days
aHUS genetic panel: CLIA method validation:

Platform-specific:
Analytical Sensitivity: >99%
Analytical Specificity: >99%

- ACMG guidelines require a concordance of 95-98% or greater. (Rehm, HL et al. Genetics in Medicine, 2013)

Disease-specific:
Clinical Sensitivity: >70% (of all true cases of aHUS)
Clinical Specificity: >99%
Study Design:

• 60 patients suspected of having aHUS were referred for genetic testing. (April 2014 – December 2014)

• The exonic regions of the following genes were sequenced: CFH, MCP(CD46), CFI, C3, CFB, CFHR1, CFHR3, CFHR4, CFHR5, Thrombomodulin (THBD), DGKE, Plasminogen (PLG).

• Data was the dis-identified and aggregated.
Study Results:

- Novel mutations were found 17% (10/60) patients suspected of having aHUS.
- 35% (21/60) patients had either a disease-associated mutation or a mutation of unknown significance.
- 65% (39/60) patients were considered negative with only polymorphism or incidental variants identified.
- The mutation frequency within genes contained in the panel was similar to previously published reports.
- 1 novel variant produced a nonsense (stop codon) in CFH.
- 7 novel variants were either in ‘hot spots’ for pathogenic mutations, created AA charge change or AA size change.
Study Results:

Gene rank order of novel variants detected when sequencing complement and coagulation genes implicated in complement-mediated TMA. (n=60)

<table>
<thead>
<tr>
<th>Gene representation</th>
<th>Percent of all results</th>
<th>Percent of non-negative results</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFH</td>
<td>5</td>
<td>17%*</td>
</tr>
<tr>
<td>CFI</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>PLG</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>MCP(CD46)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>THBD</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>CFB</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>DGKE</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CFHR 1, 3, 4, 5</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* Novel variants, † All patients tested, ‡ Previously described disease associated variants
Novel Mutation Discussion:

• We expect to see novel mutations in 0.1% (1 in 1,000) of the normal population when looking at the 12 genes in the aHUS panel.
• Seeing novel mutations occur at a rate of 17% in our aHUS referred patient population is very suspicious.
• de novo mutations occur at a rate of $1.20 \times 10^{-8}$ per nucleotide per generation. (Kong, A. 2012 *Nature*)
• Some novel mutations may be unreported rare variants, likely due to ethnicity.
Study Conclusions:

- Novel variants play an important role in the diagnosis and characterization of aHUS.
- Rapid aHUS genetic testing allows for ‘ruling in’ and confirming of the clinical diagnosis during initial hospital presentation.
- Knowing which gene contains the mutation will inform treatment decisions, transplantation decisions, others.
- Genetic testing will not ‘rule out’ a diagnosis of aHUS.
How are clinicians using the aHUS genetic panel?

1. Likely diagnosis but Institution/Insurance balking
2. Known diagnosis, considering transplant
3. Known diagnosis, patient wants to stop therapy?
4. Unclear TMA, progressive Renal Disease
5. C3 Glomerulopathy (with/without preceding TMA)
6. Emergent cases (need answer in 2 days)
   - Complement genetics reflexively after detectable ADAMTS-13
Updated Results:

• 150 suspected aHUS patients sequenced and added to our database. (~12 months)
• 42% were either positive or equivocal for aHUS-associated mutations.
• 58% were negative with only polymorphisms or incidental variants identified.
• The rank order of gene frequency for identifying a mutation is as follow:
  • CFH (14 mutations identified)
  • PLG (11 mutations identified)
  • CFI (10 mutations identified)
  • CFHR1-CFHR3 del (10 mutations identified)
  • C3, CFB, MCP (CD46) (6 mutations identified, each)
  • THBD (5 mutations identified)
  • DGKE (2 mutations identified)
What’s next:

- Gathering data for functional confirmation of novel and other mutations.
- Identify groups for collaboration on case reports.
- Adding additional genes to our panel.
Scientific and Operations Team:

Mike Ero, MT, CLS, MBA (Laboratory Director)
Brad Lewis, MD (Medical Director)
Jamey Kain, PhD (Senior Clinical Research Scientist)
Alex Joyner, PhD (Clinical Bioinformatics Scientist)
Connie Ng, CLS (Clinical Laboratory Scientist)
Bjorn Stromsness, BS (Director of Client Services)